THE T2 MYSTERY

T2 is a virus which dissolves bacteria. Normally its attack is followed by the appearance of a generation of new viruses. But sometimes the viruses appear to be missing. Why?

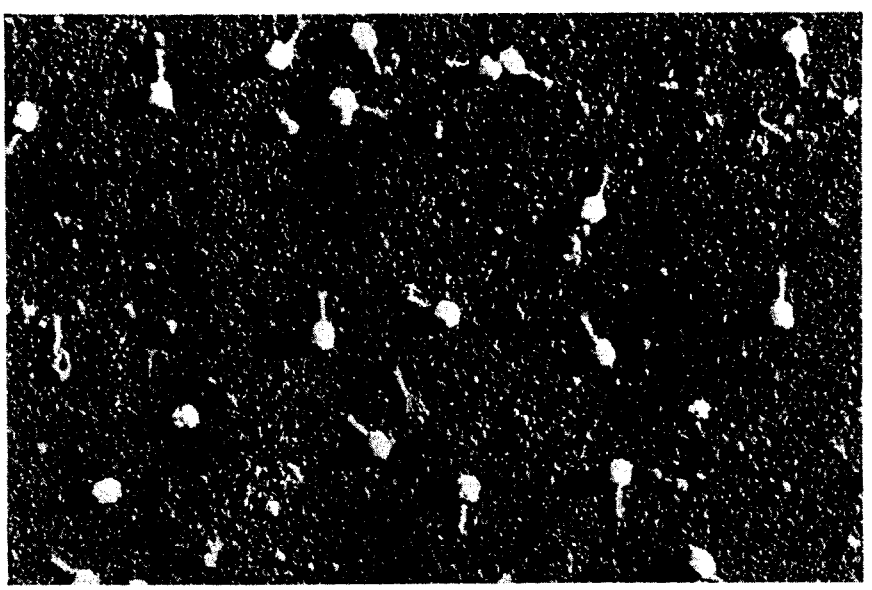
by Salvador E. Luria

Our story has as its critical episode one of those coincidences that show how discovery often depends on chance, or rather on what has been called "serendipity"—the chance observation falling on a receptive eye. The episode is a good illustration of the principle of "controlled sloppiness," which states that it often pays to do somewhat untidy experiments, provided one is aware of the element of untidiness. In this way unexpected results, sometimes real discoveries, have a chance to come up. When they do, we can trace their cause to the untidy, but known, features of the experiment.

The story has to do with bacteriophages, or bacterial viruses. The habits and reproductive cycle of these bacteria-infecting viruses are familiar to the readers of SCIENTIFIC AMERICAN. A virus particle attaches itself to a susceptible bacterium and injects its reproductive material, mainly nucleic acid; this material multiplies in the bacterial cell, and within half an hour the bacterium dissolves and out come hundreds of new mature virus particles.

In 1946, while experimenting with infection of the common colon bacterium *Escherichia coli* by the bacterial virus called T2, I noticed a peculiar violation of the usual pattern of events. Certain mutant strains of the bacterium took up the virus, were duly dissolved after the customary period but produced no detectable viruses! When the material was tested, no trace of infectious virus could be found in it. I explored this phenomenon a little further, but after playing around with it for a few weeks and getting nowhere, I shelved it in my mental files as the "T2 mystery."

In 1950 I returned to the problem. I had become interested in the study of incomplete virus particles as possible precursors of viruses, and it seemed that the juice from the bacterial mutants might be a good place to look for such precursors—arrested viruses, as it were. I proceeded to re-examine the matter with a co-worker, Mary Human.



T2 VIRUSES have polygonal heads and short tails. In this electron micrograph, made by A. E. Vatter of the University of Illinois, the virus particles are enlarged 70,000 diameters.

One day, in preparation for more complicated experiments, we decided to add some streptomycin to the juice from the dissolved bacteria. To carry out the measurements we planned to make, we needed bacteria resistant to streptomycin. It happened that no streptomycin-resistant culture of *Escherichia coli* had been prepared in the laboratory that day. Rather than wait, Mrs. Human decided to use an available streptomycin-resistant culture of another bacterium which is susceptible to T2: namely, the dysentery bacillus (*Shigella dysenteriae*). Of course the substitution made it not a "clean" test. But since virus T2 seemed to behave alike on both hosts, it hardly seemed to matter.

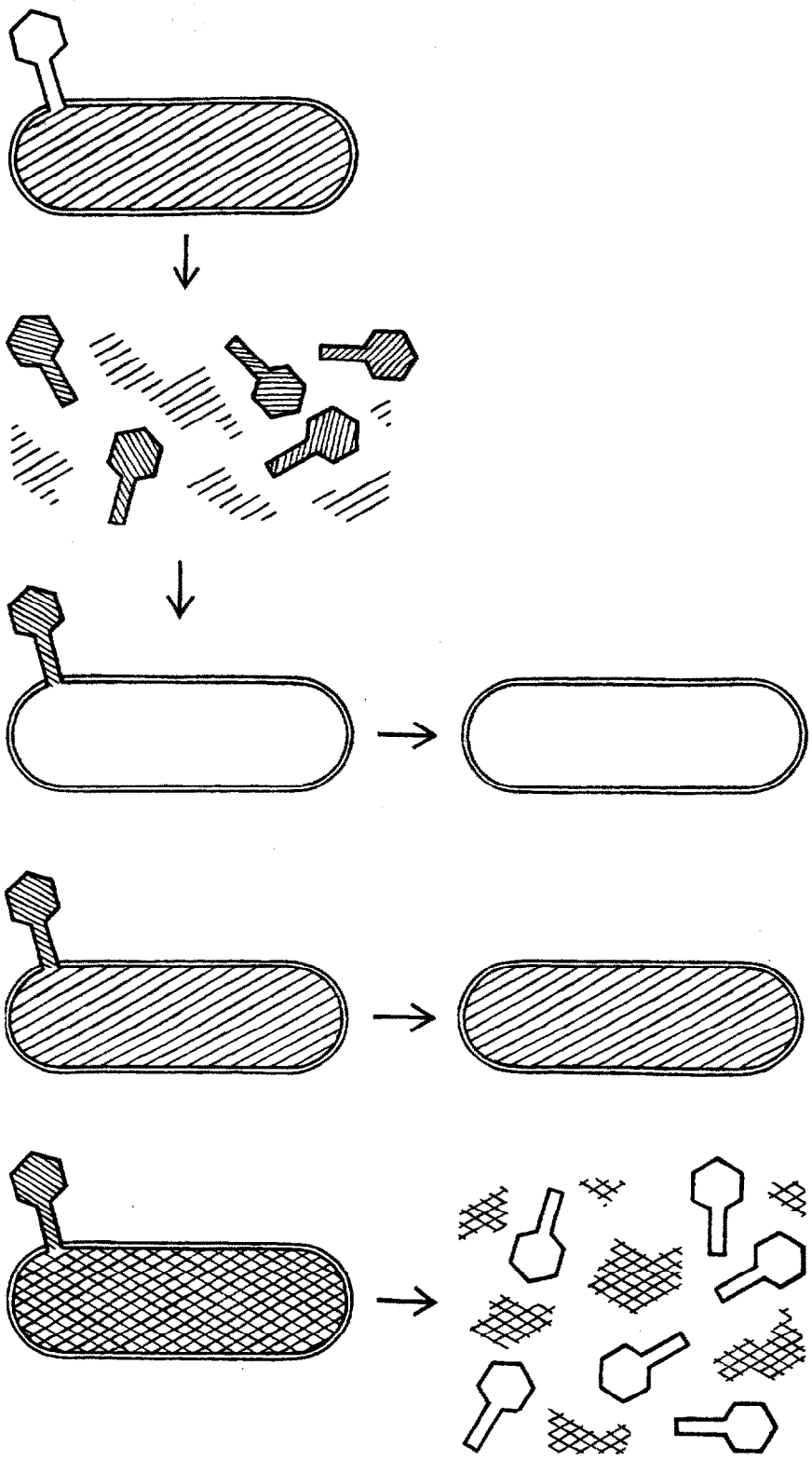
The next day the T2 mystery was solved; or rather, as often happens in science, it had been transformed into a bigger one. The juice from the dissolved coli bacteria, which had seemed virus-free, raised havoc with the dysentery bacilli. In other words, it contained plenty of infectious virus, but the virus was infectious only to the dysentery bacteria not to the coli. The mutant coli cells in which the virus had reproduced had changed it somehow. But the change was not profound: we discovered immediately that after a single cycle of reproduction in the dysentery bacilli, the virus reverted to the original T2 type—that is, it could infect coli again!

This was a great surprise. If the virus had undergone a stable, hereditary

change during reproduction in the unusual, mutant coli, that would have been understandable. It is not uncommon, when a virus invades a new host, for a mutant type of virus to emerge and become dominant. In that case the host has simply favored mutant viruses which happen to be present; it has not itself modified the virus. But no mutation was involved in the change of the T2 virus to the new type and back. Every T2 particle multiplying in mutant coli produced only progeny of the modified type, and every virus of the modified type gave only progeny of the original T2 type when it reproduced in dysentery bacilli. What we had, in short, was a nonhereditary modification of the virus imposed by the host bacterium itself.

Within a few months workers in many laboratories found cases of host-induced modifications in all sorts of bacteriophages besides T2. There was one important difference, however. The modification of T2 is "nonadaptive;" that is, the modified virus cannot grow in the host that changed it. In most of the other cases the changes are adaptive: the changed virus can grow in the host that modified it but becomes unable to grow in a second host, and when occasionally a particle manages to overcome the restriction against growing in the second host, it immediately gives rise to fully adapted particles. Return to the first host erases the adaptation completely. The virus has no "memory" of any host but the very last. Each modification eliminates all the previous ones.

The discovery of the ability of bacteria to alter their parasites raised a number of questions. First of all, what property of a bacterium gives it this power? Clearly the answer lies in the genetic make-up of the bacterium. A single mutation in the common coli bacterium, for instance, transforms it into the mutant variety that modifies the T2 virus. A most remarkable thing is that viruses themselves sometimes bestow the virus-modifying property on bacteria. There is a latent form of virus known as "provirus," or "prophage" [see "The Life Cycle of a Virus," by André Lwoff; SCIENTIFIC AMERICAN, March, 1954]. The prophage, apparently incorporated in the chromosomes of the host bacterium and multiplying with them, occasionally turns into full-fledged virus and destroys the bacterium. Some prophages control the production of substances by their hosts (*e.g.*, diphtheria toxin) or have other important effects on them. Now two British bacteriologists, E. S.

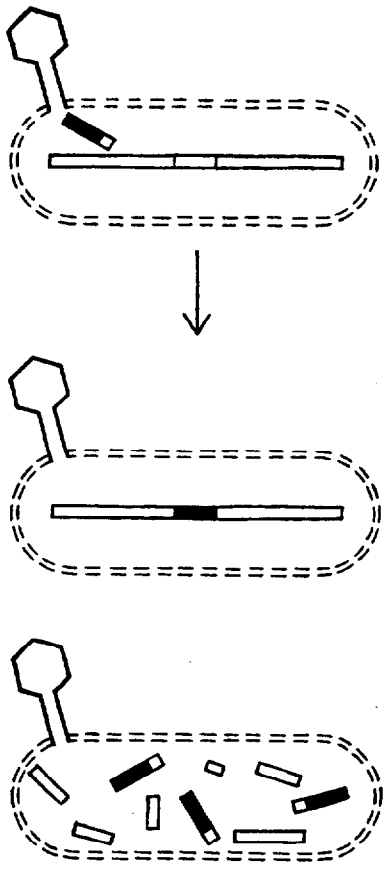


MYSTERY IS EXPLAINED by this diagram. A normal T2 virus infects a mutant variety of the bacterium *Escherichia coli* (*first horizontal row*). The bacterium dissolves, liberating not normal viruses but modified ones (*second row*). When one of these modified viruses attacks a normal bacterium (*third row, left*), the bacterium dies but no new viruses appear (*third row, right*). When a modified virus attacks a mutant bacterium (*fourth row, left*), the same thing happens (*fourth row, right*). When a modified virus attacks the entirely different bacterium *Shigella dysenteriae* (*fifth row, left*), the bacterium dissolves and liberates normal viruses like the one which attacked *Escherichia coli* (*fifth row, right*).

Anderson and A. Felix, have discovered that a prophage can cause certain typhoid bacilli to produce modifications in viruses completely unrelated to the prophage.

In our laboratory Seymour Lederberg has discovered recently that a single virus particle can possess two distinct host-induced modifications. The virus is first modified so that it can grow in a host in which it could not grow before. A second modification enables it to grow in a host containing a certain prophage. Both adaptations are reversible: they can be removed by letting the modified virus reproduce a new generation in an appropriate normal host.

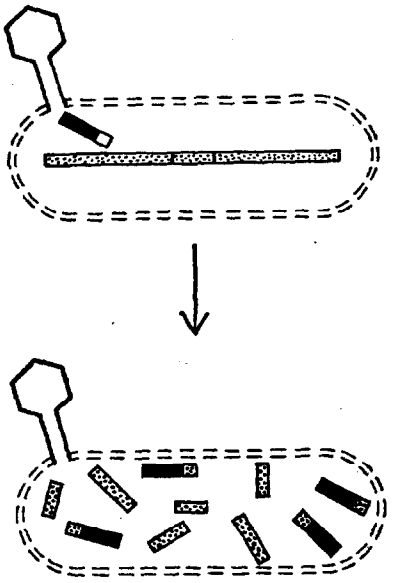
These findings prove that a bacte-



MYSTERY IS INTERPRETED at the level of the chromosome. A normal virus injects its nucleic acid into a normal bacterium (*top*). The viral nucleic acid is represented by the short rectangle; the bacterial chromosome, by the long one. The short rectangle is divided into two parts. One of them (*white*) can be modified separately by the bacterium. The segment in the middle of the bacterial chromosome is the region at which it is attacked by the viral nucleic acid. Normally one of two things might happen. The viral nucleic acid might incorporate itself into the chromosome as "prophage" (*middle*). Or the viral nucleic acid might reproduce itself and destroy the chromosome (*bottom*).

rium's modifying influence on a virus can be traced to specific portions of the host's hereditary material. Indeed, the prophage-controlled properties of bacteria may become extremely useful in the study of latent viruses and of gene action in general.

Exactly what are the changes that occur in a modified virus? We still do not know, but we can guess where to look for the differences between the original and the altered virus. The critical stage in the life cycle of a bacterial virus comes just after its hereditary material, the nucleic acid DNA, invades the bacterium. There is a good deal of circumstantial evidence that the injected virus material ordinarily establishes some contact with the nucleus of the host cell. There it takes one of two courses: it may become integrated with the host nucleus as prophage or it may begin at once to reproduce as virus. Now when a virus is modified in such a way that it cannot grow in a certain host, the halt in its development comes at this early stage. The virus's reproductive material penetrates into the host, but somehow it fails to make the proper adjustments for reproduction. It neither reproduces nor becomes prophage. The guess is that this failure is due to a change in the virus's nucleic acid which prevents it from establishing fruitful contact with the nuclear material of the host. One piece of evidence which may support this concept is that some modified viruses



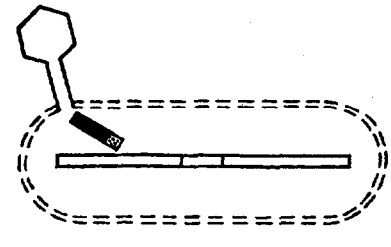
NORMAL VIRUS injects its nucleic acid into a mutant bacterium. Here one part of the viral nucleic acid (*stippled*) might be modified by the bacterium. Thus the bacterium would liberate modified viruses.

can be made to grow in unreceptive hosts by pretreating the host cells with ultraviolet light, which acts rather specifically on their nuclear apparatus and may facilitate successful contact.

Is it possible that modifications like those in bacterial viruses may occur in the viruses responsible for human diseases? We have no way of knowing so far; indeed, there is no evidence that the multiplication of viruses in animal cells is at all like the reproduction of bacterial viruses. Yet the Australian virologist H. J. F. Cairns has observed a suggestive parallel. When influenza virus grown in a chicken egg is transferred to the brain of a mouse, it multiplies only in the first batch of cells that it meets and no further. Cairns suggests that the brain cells may modify the virus in such a way that it ceases to be able to grow in such cells, though the modified virus can still grow in eggs—just as the modified T2 virus becomes unable to grow in the coli cells that produced it but can multiply in the dysentery bacillus.

This gives rise to some interesting speculations. If animal cells can modify viruses, they might well control the spread of viruses in animal tissues. Some viruses that have multiplied in certain organs can be stopped by others. We may even speculate about the possibility that there are viruses which transform normal cells into tumor cells and then are so modified themselves in the latter that they cannot reproduce further.

A new view of the nature of viruses is emerging. They used to be thought of solely as foreign intruders—strangers to the cells they invade and parasitize. But recent findings, including the discovery of host-induced modifications of viruses, emphasize more and more the similarity of viruses to hereditary units such as genes. Indeed, some viruses are being considered as bits of heredity in search of a chromosome.



MODIFIED VIRUS injects its nucleic acid into a normal bacterium. Here the modified viral nucleic acid is unable to make fruitful contact with the chromosome. Accordingly the virus does not reproduce.